To:	BLA STN 103951
From:	Gary Kikuchi and Elizabeth Shores e.g. & Shores 8/29/01
Through:	Fred Mills DTP, Barry Cherney Deputy Director DTP, Amy Rosenberg, Director DTP Barry Cherney 8/29/01
Date:	August 29, 2001

Immunogenicity Review of ARANESP (Amgen, for anemia in patients with chronic renal failure)

I. Introduction/Administrative issues:
Product: ARANESP (ARANESP), or Erythropoiesis Stimulating Protein (Amgen)
Indication: treatment of anemia in patients with chronic renal failure.
Reviewers

Primary/chair: Fred Mills
Addition Product Reviewers: Serge Baucage, Elizabeth Shores, and Barry Cherney.
Pharm/Tox: Mercedes Serabian, pharm/tox
Clinical Reviewers Ellis Unger, Marc Walton, DCTDA branch chief
CSO: Lori Tull, CSO.

II. Summary

A. Product Overview: ARANESP is a hyperglycosylated analogue of recombinant human erythropoietin. 5 amino acid changes have been inserted into the native molecule, encoding 5 N-linked carbohydrate side chains rather than the 3 N-linked carbohydrate chains on the native molecule. In the BLA, data are presented indicating that this change increases the serum half-life of the molecule. ARANESP is therefore indicated for less frequent dosing than erythropoietin. ARANESP stimulates erythropoiesis by the same mechanism as erythropoietin. Because the product is slightly altered from endogenous erythropoietin, it has the potential to induce an immune response not only to itself (ARANESP) but to the endogenous protein. Such an "autoimmune response" could have important and severe implication for the health of treated patients as discussed below.

B. Detection of anti-NESP antibodies This review focuses on the immunogenicity (induction of antibody response) of ARANESP. In this BLA, two antibody detection assay are described.

1. A [redacted] assay [redacted] was performed to initially screen samples from the patients receiving the product, ARANESP (Erythropoiesis Stimulation Protein) for anti-NESP antibodies. This assays employs [redacted]
2. An ARANESP [redacted] assay, was designed to be used for samples

that scored positive in the original screening assay. This assay employs a [redacted]

Antibodies were not detected by the screening assay [redacted] in 1533 patients studied in clinical trials presented in this BLA. Hence the [redacted] was not employed to examine patient samples However, it remains a concern that the screening assay is not sensitive enough to detect antibodies in humans.

C. Issues with antibody detection assays.

1. Initial Reported Sensitivity of the [redacted] assay. The limit of detection of the [redacted] screening assay was initially reported as [redacted] ng/ml of antibody in human serum. CBER reviewers were concerned about the poor sensitivity of this assay. These concerns were relayed to the company in the CR letter of February 16, 2001 (attachment 1) Based on this initial review of the BLA, post-marketing commitments were obtained for the design of new assays with improved sensitivity as discussed in a telecon of March 26 2001 (attachment 2). These new assays should address concerns regarding sensitivity of the screening assay and the limitations of the original assay to detect immunoglobulin isotypes such as IgM, IgA, IgE, and IgG3.

   During discussions of these new assays on March 26/29 (attachment 2 and 3), the sponsor stated that the original assay described in the BLA had a sensitivity of [redacted] ng/ml of antibody in human serum, not [redacted] ng/ml as previously stated. However, original data was not provided to support this contention. The sponsor was asked to submit data sets supporting a sensitivity of [redacted] ng/ml but the data were not received during the review period. Data regarding sensitivity of the [redacted] assay will be submitted along with other data to fulfill the post-marketing commitments.

2. Issues related to antibody detection assays using [redacted]
   a. [redacted] does not or poorly binds certain antibody isotypes, including IgM, IgA, IgE, and IgG3. Therefore, IgM antibodies, the first antibody generated during an immune response would not be detected making this assay ineffective for revealing early immune responses
   b. Anti-ARANESP antibodies of isotypes that fail to bind [redacted] may interfere with detection of other antibody isotypes.
   c. Validation of assay methods are performed by using [redacted] anti-ARANESP (or anti-epo) antibodies. If [redacted] antibodies have different affinities for [redacted] it may be difficult to extrapolate the sensitivity of assay performed with [redacted] antibodies to those of human antibodies.

D. Implications of anti erythropoietin antibodies.

There have been documented clinical cases of antibodies to recombinant erythropoietin and rare cases have been reported in association with pure red cell aplasia. It is essential to ensure that the immunogencity assays are sufficiently sensitive to assure that pathogenic levels of antibodies to ARANESP are not achieved. Because of the

potentially severe nature of these cases of pure red cell aplasia associated with erythropoietin and because of the limited clinical experience using ARANESP (<2000 patients), a class warning will be requested in the package insert.

E. Implementation of antibody detection assays.

1. Primary screen. In this assay, patient serum samples are incubated with [redacted] ARANESP, then with [redacted] and then [redacted]

2. Secondary assay. The secondary assay is a [redacted] in which [redacted] ARANESP. The potential for patient serum samples to inhibit the activity of this assay is assessed.

3. Decision point analysis. Decision point analyses for invoking the secondary screen based on positive results of the primary screen are discussed below.

III. Primary screening assay [redacted]

A. Method

The [redacted] assay was designed to detect antibodies to rhEPO or ARANESP in human serum. This assay is [redacted]

amount of [redacted] is directly proportional to the amount of total antibody (Ig) bound.

B. Elements of [redacted] Primary Screening Assay Validation

Information was provided in the BLA to validate the precision (intra-assay and inter-assay), accuracy, sensitivity, specificity, linearity, and ruggedness of the primary screening assay.

[redacted]

[redacted]

Critical Comment. *(As discussed in the summary above) Because a [redacted]-fold dilution of antibody is used in clinical samples, the limit of detection of the [redacted] assay was originally calculated to be [redacted] ng/ml. This number is indicated in the original CR letter. However, in subsequent discussions, the sponsor indicated that the [redacted] assay was sensitive to [redacted] ng/ml. No additional data was submitted to substantiate those statements. In those subsequent discussions, the sponsor also indicated that the [redacted] assay, as discussed above, had reduced capability to detect isotypes such as IgM, IgA, and IgE. For these reasons, post-marketing commitments were undertaken.*

4. Specificity

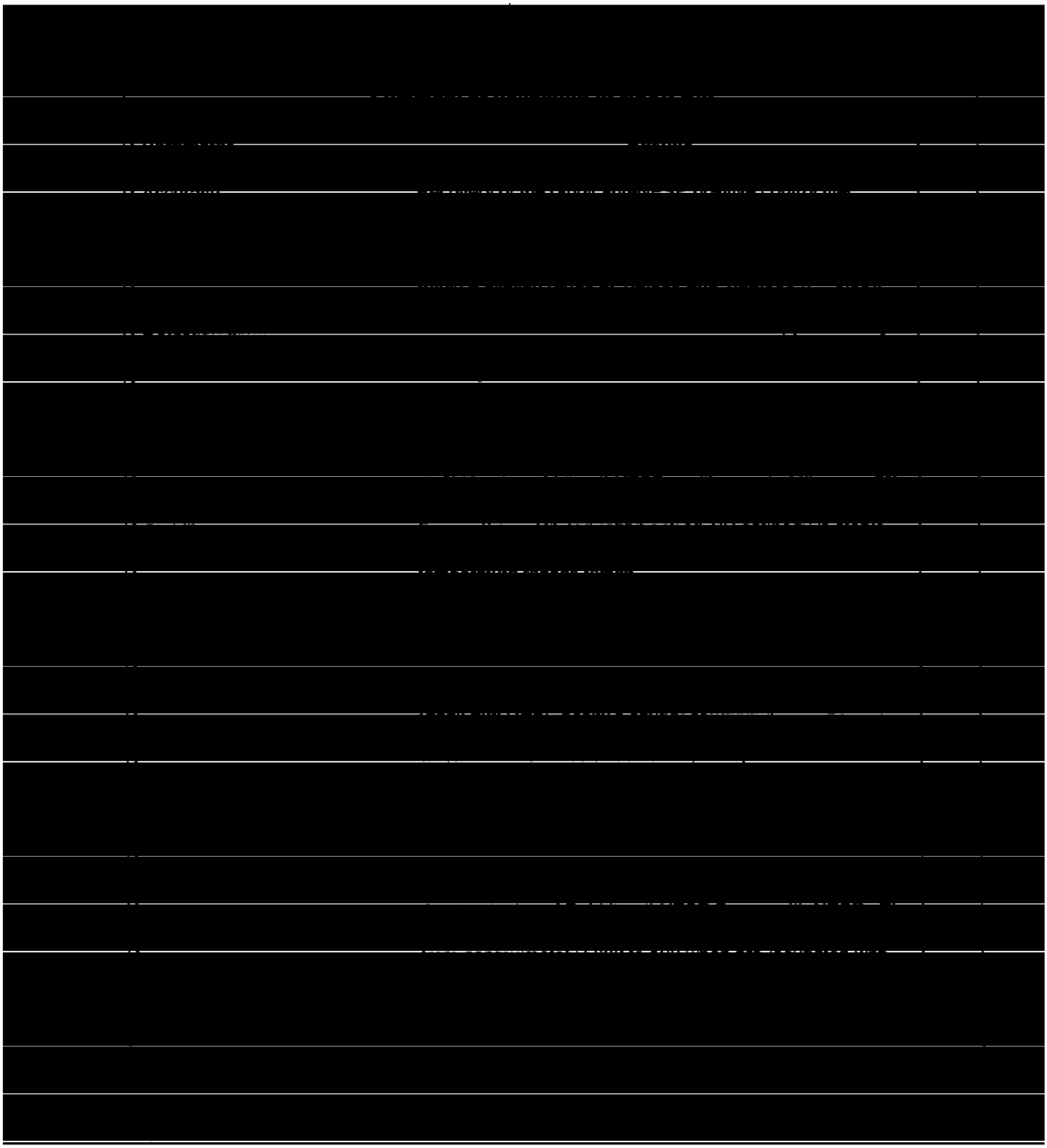
[redacted]

[redacted]

Comment. With regard to specificity claims, no original data are provided, so it is difficult to verify the claims of specificity. The claim that rh EPO did not interfere with the assay, which is made in the Integrated Summary of Safety, is not mentioned in the text of the validation report captured above.

5. Linearity. [redacted]

[redacted]



V. Decision point analysis.

A. Implementation

In the screening assay, serum samples were analyzed for seroreactivity to ARANESP or r-HuEPO using a [redacted] assay. A sample was considered reactive if the [redacted] were [redacted] times the predose value in [redacted] independent screening assays. Using a second aliquot of the sample that tested reactive, the screening assay would then be repeated. Confirmation of seroreactivity would then be established by repeating the above procedure on a serum sample obtained from the subject at a later time point. If the repeat sample is also seroreactive, both samples (and the predose control) would be tested using the ARANESP [redacted] Assay to determine whether [redacted] antibodies are present. The [redacted] assay is based on [redacted] of a ARANESP- and [redacted] A sample would be considered inhibitory if the serum [redacted] independent assays.

B. Implications for Phase IV Commitments

The decision point analysis is well described. However, a new decision point analysis with adequate justification will need to be provided with the new assays submitted to fulfill the post-marketing commitments.

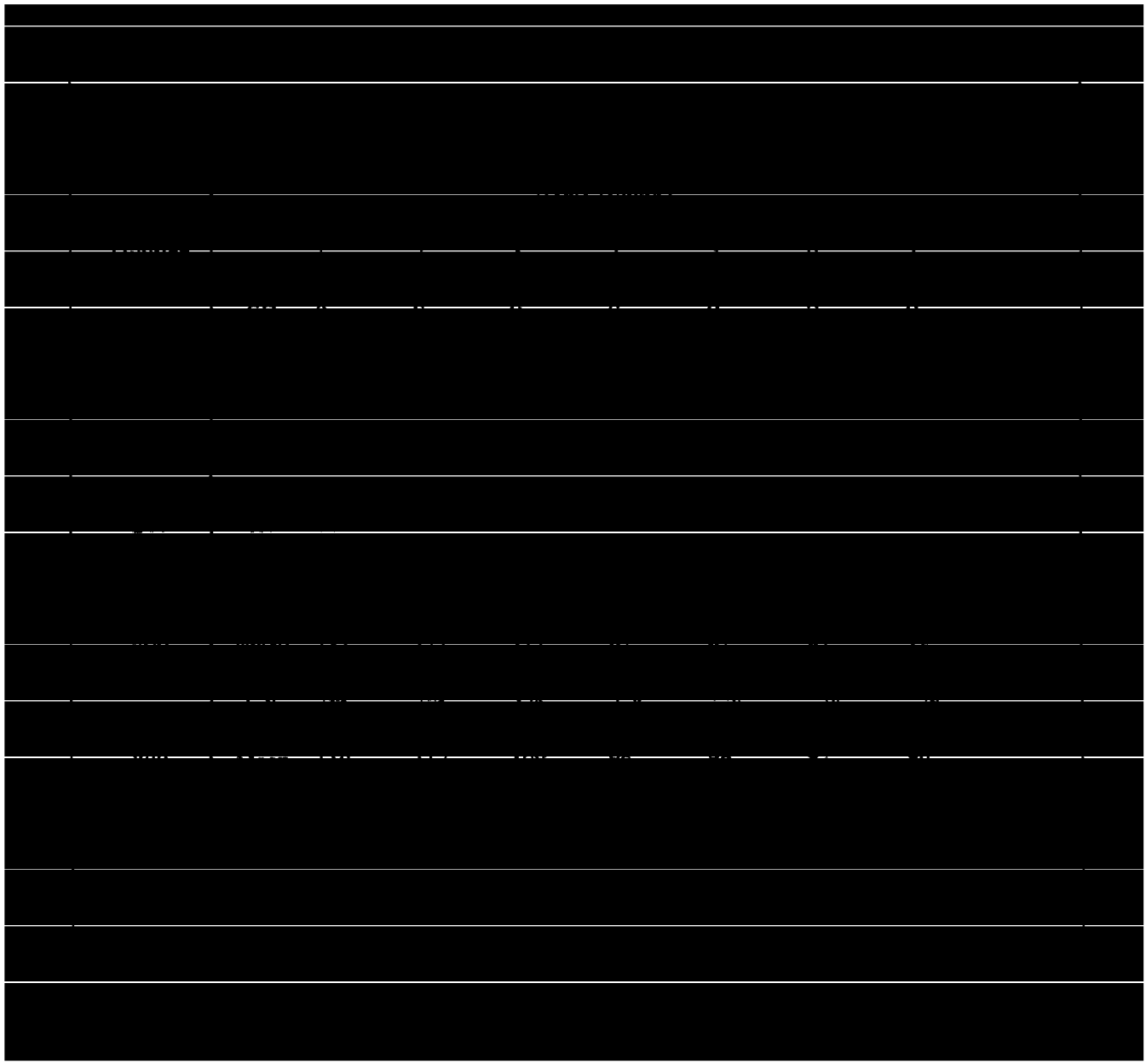
VI. Secondary [redacted] Assay

A. Method.

The [redacted] Assay was developed to detect neutralizing antibodies in vitro by measuring the [redacted] The [redacted] response to ARANESP (2 ng/mL) is used as the [redacted] control. Test samples are diluted [redacted] in assay matrix prior to analysis. [redacted] dilutions of the [redacted] positive control are used to generate a dose response curve and test samples are analyzed in conjunction with the [redacted] positive control. Each sample and control is added to [redacted] in a [redacted] format and incubated for [redacted] is added to each [redacted] and incubated for an additional [redacted] After incubation, [redacted] and percent of [redacted] are determined from the [redacted]

B. Validation of the secondary [redacted] assay.

1. Precision – intra-assay. [redacted]



2. Precision – inter-assay. [redacted]

[redacted]

3. Accuracy, intra-assay. [redacted]

4. Accuracy, inter-assay. [redacted]

5. Assay sensitivity. [redacted]

[redacted]

6. Assay Specificity

[redacted]

b. Assay Specificity: [redacted]

[redacted]

C. Assay Specificity: [redacted]

d. Assay Specificity: [redacted]

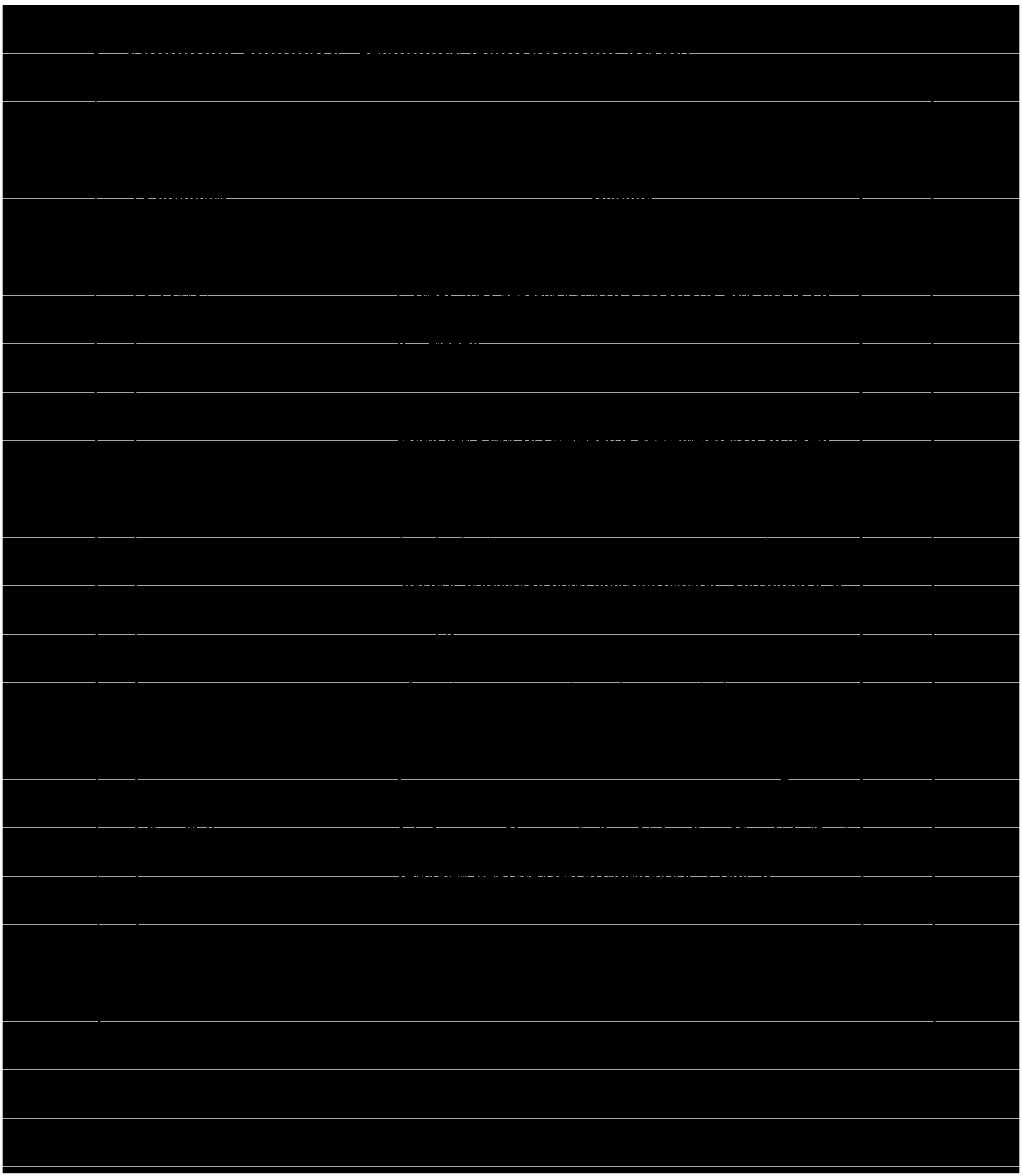
7. Linearity. [redacted]

8. Ruggedness. [redacted]

9. Interanalyst effect. [redacted]

[redacted]

Stability – Freeze-thaw. [redacted]



VII: Overall clinical results and implications of immunogenicity assays.

A. **Patient population:** This BLA contains data on use of ARANESP for treatment of anemia associated with chronic renal failure. Patients receive ARANESP in doses starting at approximately 0.45 micrograms/kg IV 1 time per week on a regular basis. This is a chronic exposure to the product. Since patients receiving ARANESP have expressed the gene for native erythropoietin at some point since birth, they are anticipated to be immunologically tolerant to erythropoietin.

B. **Timing of antibody sampling:** The median number of weeks between the first dose and last antibody sample was 25 weeks for ARANESP, with a range of 1 to 115 weeks, and 29 weeks for EPO with a range of 2 to 71 weeks.

C. **Results of immunogenicity assays.** In the BLA, an extremely low rate of incidence of antibodies to ARANESP was observed. This is summarized in the following table.

Table IVC-7.
Antibody Assay Results (Safety Analysis Set)

	NESP	r-HuEPO
Number of Subjects	1578	591
Number of Subjects with Antibody Assay Results[a]	1534	572
Screening Assay[b]		
Seronegative	1533 (100%)	572 (100%)
Seroreactive	1 (0%)	0 (0%)

Note: Percentages are based on number of subjects with antibody assay results Page 1 of 1
[a] Excludes 2 r-HuEPO subjects who received NESP during study
[redacted]

The text accompanying this table states: One subject in the ARANESP group (Subject 140042034) had a single reactive sample at week 24, with negative results obtained at weeks 12, 36, and 38. As per the prespecified antibody assay protocol, no further characterization (such as testing in the [redacted] antibody assay) was conducted, since the seroreactivity was not confirmed in [redacted] independent samples. This subject experienced no decrease in hemoglobin or unusual adverse events. To summarize, this patient is not considered seroreactive.

As noted above, since all of these results were essentially negative, th ARANESP [redacted] antibody assay was not performed.

D. **Important concerns regarding potential immunoreactivity of erythropoietin.** Some cases of patients developing immunological reactions to recombinant erythropoietin have been reported. One patient presented these symptoms after treatment with erythropoietin in the United States (Prabhakar and Muhlfelder,

Nephrology (1997) 47: 331). Other cases have been reported in Europe. In one of these cases, the patients presented with resistance to administration of recombinant erythropoietin (Peces R. et al., NEJM (1996) 335: 523-524.) In another case, a patient presented with symptoms of anaphylaxis (Garcia JE et al., Nephron (1993) 65: 636-637.) Generally elevated levels of antibodies to erythropoietin in a large panel of patients receiving recombinant erythropoietin was reported (Castelli G. et al., Pharmacological Research (2000) 41: 313-318.) An abstract from the American Society of Hematology reports additional patients with antibodies against human erythropoietin and the clinical syndrome pure red cell aplasia (Casadevall P et al., Blood 1999 94: 50a abstract #211). Finally, elevated levels of antibodies to erythropoietin have also been reported in HIV-1 related anemia (Sipsas N.V. et al., J. Infectious Diseases (1999) 180: 2044-2047), as well as patients with systemic lupus erythematosis (Tzioufas AG et al., Arthritis Rheum (1997) 40: 2212-2216.)

Although these cases appear relatively rare, the presence of these reports suggests that antibody responses are possible. Surveillance of antibody responses to recombinant erythropoietin is essential to assure product safety. Because of the serious nature of pure red cell aplasia, it was considered necessary to insert a class warning in all products in the erythropoietin class.

E. Final Approved Label

Background: CBER remains concerned as to immunogenicity of NESP and related products and the development of red cell aplasia (as per telecon of July 26 (attachment 5, and sited literature). Moreover, CBER remains concerned about the sensitivity the antibody detecting assays used in the BLA trial, the final labeling (as discussed throughout the review). Therefore, the final labeling regarding immunogenicity was composed and accepted by Amgen as per telecon of August 21, 2001 (attacment 6).

"Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. The incidence of antibody development in patients receiving Aranesp™ has not been adequately determined. Radioimmunoprecipitation and neutralizing antibody assays were performed on sera from 1534 patients treated with Aranesp™. High-titer antibodies were not detected, but assay sensitivity may be inadequate to reliably detect lower titers. Since the incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay, and the observed incidence of antibody positivity in an assay may additionally be influenced by several factors including sample handling, concomitant medications, and underlying disease, comparison of the incidence of antibodies to Aranesp™ with the incidence of antibodies to other products may be misleading.

Erythrocyte aplasia, in association with antibodies to erythropoietin, has been reported on rare occasions in patients treated with other recombinant erythropoietins. Due to the close relationship of Aranesp™ to endogenous erythropoietin, such a response is a theoretical possibility with Aranesp™ treatment, but has not been observed to date.

There were no reports of serious allergic reactions or anaphylaxis associated with Aranesp™ administration among the 675 patients receiving Aranesp™ for more than 6 months in clinical trials. If an anaphylactic reaction occurs, Aranesp™ should be immediately discontinued and appropriate therapy should be administered.

VIII. Post-marketing commitments

Amgen agrees to improve immunogenicity assays of darbepoetin alfa according to the following schedule:

a. Evaluation of improved methods for detecting antibodies to darbepoetin alfa. The results of the evaluation and validation data for any improved assays will be submitted to CBER by [redacted]

b. Analysis, using the improved and validated assay, of archived serum samples on 500 CRF patients who have been treated with the ARANESP albumin formulation and on 1000 CRF patients who have been treated with the ARANESP polysorbate formulation. The results, and any necessary revised labeling, will be submitted to CBER by [redacted]

c. If antibodies to darbepoetin alfa are detected, Amgen commits to submit data establishing whether antibodies to darbepoetin alfa cross-react with native erythropoietin.

In addition, immunogenicity reviewers (Gary Kikuchi, Elizabeth Shores, Amy Rosenberg), think it important to have an interim discussion with the company to ensure the sponsor is unambiguously aware of CBER concerns regarding the present assay and implication for clinical use.

Attachment 1

Comments to the sponsor that were transmitted in the CR letter of February 16, 2001, regarding immunogenicity of ARANESP (Erythropoiesis Stimulating Protein) Amgen:

1. The current assay for antibodies to ARANESP does not appear sensitive enough, because taking into account the dilution factor, the assay can only detect antibodies to ARANESP at a threshold level of [redacted] ng/ml. Given the level of sensitivity of the assay, it is not possible at the present time to assess the incidence of antibody formation to ARANESP in patient samples. To remedy the problems regarding sensitivity and quantitative capacity of your assay, we request that you design a new assay for anti-ARANESP antibodies with more sensitive detection levels, and of proven quantitative ability. We further request that you archive current serum samples for testing in future assays.

2. Should the new assay detect antibodies to ARANESP in patients, it will be critical to establish whether they neutralize ARANESP and cross-react on native EPO. The [redacted] antibody assay that you have developed demonstrates an adequate sensitivity, specificity and quantitative ability. Please describe the assay you intend to use to assess cross-reactivity to native or recombinant human EPO.

3. You have submitted information on immunogenicity of ARANESP in a formulation containing albumin but not the polysorbate-containing, albumin-free formulation. Please provide information on the immunogenicity of the polysorbate-containing formulation of ARANESP using the assays described above.

Attachment 2

Telecon of March 26, 2001 in Response to CR letter of February 21, 2001
(This may be a retrospective paraphrasing of the conversation)

The response to the CR letter was submitted on Feburary 21 2001. This response was discussed in a telecon on March 26, 2001 The key issues with regard to immunogenicity are as follows:

Introduction. The sponsor stated that they were committed to improving the sensitivity of the assays as discussed in the CR letter, point 1 above. The sponsor agreed to address these issues and proposed a program that will be completed as a postmarketing commitment by [redacted]

Overview of Assays. The sponsor has developed four antibody assays to support the ARANESP clinical program, with sensitivities summarized but not supported by data in the supplement.

Assay	Stated sensitivity	Status
[redacted]	[redacted] ng/ml	In BLA
		In BLA

[redacted] In the February 21 submission, the sponsor stated that the [redacted] assay was very sensitive with limit of detection [redacted] ng/ml. However, the sponsor did not provide original data supporting this claim of limit of detection. The sponsor also stated that this assay was useful for identifying human IgG antibodies but less sensitive for detecting other immunoglobulin isotypes, e.g. IgM, IgE, and IgA.

Comment: In the Feburary 21 submission, the sponsor indicated that the current [redacted] *assay has reduced sensitivity to immunoglobulin isotypes IgM, IgE, and IgA. Because of this point, the post-marketing commitments have been retained despite the sponsor's claims that the* [redacted] *assay has a sensitivity of* [redacted] *ng/ml.*

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WAS DETERMINED TO BE

NOT RELEASABLE

Attachment 3

(Please note the date of March 29, 2001 may be mistaken for March 26, 2001.)

The following comments were discussed in the minutes of the telecon of March 29, 2001: The proposed phase IV commitment plan appears acceptable provided the sponsor can submit quantitative data supporting their claims of relative sensitivity. In particular, the claim of sensitivity of the [redacted] ng/ml should be supported by data which would be provided in the [redacted] amendment. Although sensitivity was the primary issue discussed in the CR letter, there are also other issues with the current assay discussed in the review, including inability to detect a wide range of isotypes and potential for interference by product within serum samples. Because of these and other issues, the phase IV commitment plan should be adhered to.

Attachment 4

April 23, 2001 telecon (Summary)

In this telecon, CBER requested additional technical information regarding some of the alternative methods being proposed for immunogenicity assay in the February 21 response to the CR letter. In some of these methods, particularly [redacted]

Attachment 5

July 26, 2001 Telecon

The focus of this telecon held with Amgen was to discuss the reports of pure red cell aplasia associated with antibodies to erythropoietin that had come to our attention in patients treated with erythropoeitin. Amgen performed the immunogenicity assays for many of these patients, which was a [redacted] assay of design similar to the [redacted] assay for antibodies to ARANESP. The information discussed in this telecon directly affects the review of immunogenicity assays.

At this telecon, there was a discussion of issues not central to the issue of sensitivity and specificity of immunogenicity assays. However, Amgen performed the immunogenicity assays on samples from patients with pure red cell aplasia associated with antibodies to erythropoietin obtained by a [redacted] In [redacted] patients, the antibody levels assayed by [redacted] assay ranged from [redacted] micrograms/ml. The antibody levels assayed by [redacted] ranged from [redacted] micrograms/ml. This is the first time that the sponsor has provided laboratory data regarding the levels of pathogenic levels of antibodies to erythropoietin that relate to assay sensitivity.

There are two outstanding concerns with respect to this problem. The first is that there is very little information with regard to baseline and subclinical levels of antibodies in patients that later develop pure red cell aplasia with antibodies to erythropoietin. There is therefore no information regarding the required sensitivity of immunogenicity assays as surveillance for the clinical condition, rather than use of this assay for confirmation for patients that already present with clinical symptoms. The assay must have adequate sensitivity for surveillance, not merely for confirmation. The second is that the incidence of this complication is extremely rare. It is therefore possible that surveillance of numbers of patients treated in clinical trials (approximately 1500) may not yield any positive samples, and that surveillance on a much larger scale is necessary to yield meaningful results.

Taking into consideration the above concerns, a class warning regarding the possibility of pure red cell aplasia with antibodies to erythropoietin for erythropoietin of all classes was inserted in the labeling for ARANESP.

Attatcment 6

To: BLA 99-1492/ STN 103951 (Amgen NESP)
From: Gary Kikuchi, Elizabeth Shores
Through: Barry Cherney, Deputy DTP, Amy Rosenberg, Director DTP
Date: August 22, 2001

Minutes of Telecon of August 21, 2001 with special emphasis on product and immunogenicity issues.

This telecon, which was initiated at 2:30 PM August 21, focused on clinical, product, and immunogenicity issues with regard to the package insert. Only issues relating to product and immunogenicity are included in these minutes. Clinical issues were discussed between Amgen and Marc Walton and are not covered in these minutes.

Present FDA: Marc Walton, DCTDA, Amy Rosenberg, DTP, Elizabeth Shores, DTP, Gary Kikuchi DTP
Present Amgen: Cheryl Anderson, Brad Marone, Rob Brenner, Steve Swanson, Tony Gringeri, Alan Forsyth, David Guccini, Roger Perlmutter (some names may be misspelled)

[redacted]

CBER response: *This change will need to be discussed internally, and CBER will get back to Amgen on this point.*

Sponsor: A discussion began regarding the immunogenicity section of the PI. The initial focus was on the sensitivity of the [redacted] screening assay. Amgen stated that the sensitivity of the present [redacted] assay was [redacted] ng/ml but had additional data supporting improved sensitivity of this screening assay ([redacted] ng/ml) . Amgen stated that althought they has stated the original sensitivity of the assay was [redacted] ng/ml, they had already reported to CBER that this value was an "error". They stated that they had placed the arrow on the graph at the wrong "spot". Amgen also referred to previous amendments in which original statements regarding sensitivity of the assay had been modified.

CBER response: *CBER responded by saying that datasets supporting any claims of current or modified sensitivity of the [redacted] assay would need to submitted for review. (Internal notation: the timeline for submission of the datasets supporting modified sensitivity of the [redacted] assay was not discussed during the telecon. Based on previous discussions with the sponsor, Dr. Kikuchi proposes [redacted] the milestone for the evaluation and validation data for improved assays, as the milestone for*

submission of the datasets supporting modified sensitivity. He also propose communicating this milestone with the sponsor.)

Sponsor: The sponsor remained concerned about wording in the Immunogenicity section of the PI suggesting the antibody detection assay may not have been sufficiently sensitive. Additional discussions were held regarding the wording of the immunogenicity section.

CBER response: *The wording of the immunogenicity section also hinges on other characteristics of the immunogenicity assays. What is the capability of the current screening assay, which utilizes [redacted] to detect immunoglobulin isotypes that do not bind [redacted] including IgM, IgA, and IgE? To what extent do these isotypes interfere with detection of antibodies that do bind [redacted]*

Sponsor: The current [redacted] which utilizes [redacted] has a reduced capacity to detect IgM or other isotypes compared to IgG. New assays that have increase capacity to detect these isotypes are under development. These new assays will be reported according to the post-marketing agreements described below. Based on these discussions, Amgen does not request revisions in the current PI immunogenicity section proposed by CBER.

Summary of Action items:

1. CBER will discuss the proposed changes in the DESCRIPTION section and respond to Amgen

2. Amgen will provide additional datasets supporting claims of improved sensitivity in the [redacted] screening assay. Milestones for this additional data will be discussed.

3. Postmarketing commitments regarding immunogenicity studies discussed in the 17 August fax from Amgen are agreed upon. To summarize, these are:

   a. Evaluation of improved methods for detecting antibodies to darbepoietin alfa. The results of the evaluation and validation data for any improved assays will be submitted to CBER by [redacted]
   b. Analysis, using the improved and validated assay of archived serum samples on 500 CRF patients who have been treated with the ARANESP albumin formulation and on 1000 CRF patients who have been treated with the ARANESP polysorbate formulation. The results, and any necessary revised labeling, will be submitted to CBER by [redacted]
   c. If antibodies to darbepoietin alfa are detected, Amgen commits to submit data establishing whether antibodies to darbepoetin alfa cross-react with native erythropoietin.

1. Amgen accepts the current immunogenicity section of the PI as proposed by CBER.